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(54) Title: TREATMENT OF HYPERSENSITIVITY REACTION DISORDERS

(57) Abstract

T–cell mediated delayed type hypersensitivity conditions in mammalian patients are alleviated by a process in which an aliquot of blood is withdrawn from the patient, treated extracorporeally with a combination of UV radiation and an oxidative environment, such as an oxygen/ozone gas mixture bubbled through the aliquot, and then re–injected into the patient.

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TREATMENT OF HYPERSENSITIVITY REACTION DISORDERS

FIELD OF THE INVENTION

This invention relates to the field of medicine and medical treatments. In particular, the invention relates to improved methods and compositions for treatment and prophylaxis of T-cell mediated delayed type hypersensitivity reactions in mammalian patients, involving introduction into the patient of a small amount of treated, modified mammalian blood.

BACKGROUND OF THE INVENTION

T-cell mediated delayed type hypersensitivity reactions as the term is used herein means adverse reactions on the part of a mammalian patient to foreign agents, commonly but not exclusively manifested by development of skin disorders on the patient, and in which the disorder takes at least 24 hours to exhibit full manifestation. Many of these are diagnosable by skin tests. The reactions may be chemical contact reactions, food ingestion reactions or drug ingestion reactions. Specific examples of such conditions include contact hypersensitivity reactions, in which the skin of the patient exhibits a reaction to an agent which the body has previously encountered, by contact or by inoculation. The "poison ivy" type of reaction is a specific example of contact hypersensitivity. Hypersensitivity to β-lactam antibiotics (e.g. penicillins) is an example where inoculation of a foreign agent gives rise to a skin disorder-manifested, T-cell mediated delayed type hypersensitivity. The external agents can be plant, animal, insect or reptilian secretions, chemical or biochemical antigens, from synthetic or natural sources. Various types of fibers, fabrics and the like, such as latex used in surgical gloves, can give rise to T-cell mediated hypersensitivity reaction in certain individuals. The offending external agents can be water-borne agents such as dissolved salts and minerals, encountered for example in environmental, mining, metallurgical and chemical manufacturing operations. T-cell mediated delayed type hypersensitivity

reactions are to be distinguished from psoriasis, which is an autoimmune disorder which manifests itself in red scaly skin patches having an inflammatory component, but not resulting from contact reaction.

Mammalian blood modified by exposure simultaneously to certain stressors has been reported to be useful for the treatment of a variety of pathological conditions. The stressors to which the blood is exposed are an oxidative environment namely ozone/oxygen gas mixtures applied to the blood, a temperature stressor and UV light. Thus:

U.S. Patent No. 4,968,483 Mueller et al. describes an apparatus for oxygenating blood by treating an aliquot of a patient's blood extracorporeally, with an oxygen/ozone mixture and ultraviolet light, at a controlled temperature. The apparatus taught by Mueller is proposed for use in hematological oxidation therapy.

U.S. Patent No. 5,591,457 Bolton discloses a method of inhibiting the aggregation of blood platelets in a human, a method of stimulating the immune system and a method of treating peripheral vascular diseases such as Raynaud's disease, by extracting an aliquot of blood from a patient, subjecting it to an ozone/oxygen gas mixture and ultraviolet radiation at a temperature in the range of about 37 to 43°C, and then re-injecting the treated blood in the human patient.

International Patent Application PCT/GB93/00259 Bolton describes a process for increasing the content of nitric oxide in the blood of a mammalian subject, potentially useful in treating conditions such as high blood pressure in mammalian subjects, by subjecting a sample of the patient's blood extracorporeally to three stressors simultaneously, namely an ozone/oxygen gas mixture bubbled through the blood sample, exposure to UV radiation and an elevated temperature, followed by re-injection of the treated blood sample into the patient.

International Publication No. WO 98/07436 describes an autoimmune vaccine for administration to human patients to alleviate the symptoms of autoimmune diseases such as rheumatoid arthritis. The vaccine comprises an aliquot of the subject's blood which has been subjected extracorporeally to an oxidizing environment, UV radiation and elevated temperature, simultaneously.

International Publication No. WO 96/34613 relates to treatment of vascular disorders associated with deficient endothelial function, in a mammalian subject, by administration to the patient of an aliquot of blood which has been modified by having been subjected simultaneously to stressors namely elevated temperature in the range of 37° to 55°C, ultraviolet radiation and an oxidative environment

SUMMARY OF THE INVENTION

According to one aspect, the present invention provides use for treatment or prophylaxis of T-cell mediated delayed type hypersensitivity disorders in a mammalian patient, of modified mammalian blood for administration to the patient, the blood having been modified extracorporeally by simultaneous or sequential exposure to the stress of an oxidative environment and the stress of UV radiation.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying FIGURES are presentations of the results of specific Examples described below.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

According to a preferred process of the present invention, an aliquot of blood is extracted from a mammalian subject, preferably a human,

and the aliquot of blood is treated ex vivo, simultaneously or sequentially, with the aforementioned stressors. Then it is injected back into the same subject. Preferably a combination of both of the aforementioned stressors is used.

Preferably also, the aliquot of blood is in addition subjected to mechanical stress. Such mechanical stress is suitably that applied to the aliquot of blood by extraction of the blood aliquot through a conventional blood extraction needle, or a substantially equivalent mechanical stress, applied shortly before the other chosen stressors are applied to the blood aliquot. This mechanical stress may be supplemented by the mechanical stress exerted on the blood aliquot by bubbling gases through it, such as ozone/oxygen mixtures, as described below. Optionally also, a temperature stressor may be applied to the blood aliquot, simultaneously or sequentially with the other stressors, i.e. a temperature at, above or below body temperature.

The terms "aliquot", "aliquot of blood" or similar terms used herein include whole blood, separated cellular fractions of the blood including platelets, separated non-cellular fractions of the blood including plasma, plasma components and combinations thereof. Preferably, in human patients, the volume of the aliquot is up to about 400 ml, preferably from about 0.1 to about 100 ml, more preferably from about 1 to about 15 ml, even more preferably from about 8 to about 12 ml, and most preferably about 10 ml. The effect of the combination of stressors is to modify the blood, and/or the cellular or non-cellular fractions thereof, contained in the aliquot. The modified aliquot is then re-introduced into the subject's body by any suitable method, most preferably intramuscular injection, but also including subcutaneous injection, intraperitoneal injection, intra-arterial injection, intravenous injection and oral administration.

The optionally applied temperature stressor either warms the aliquot being treated to a temperature above normal body temperature or cools the aliquot below normal body temperature. The temperature is selected so

that the temperature stressor does not cause excessive hemolysis in the blood contained in the aliquot and so that, when the treated aliquot is injected into a subject, the desired effect will be achieved. Preferably, the temperature stressor is applied so that the temperature of all or a part of the aliquot is up to about 55°C, and more preferably in the range of from about -5°C to about 55°C.

In some preferred embodiments of the invention, the temperature of the aliquot is raised above normal body temperature, such that the mean temperature of the aliquot does not exceed a temperature of about 55°C, more preferably from about 40°C to about 50°C, even more preferably from about 40°C to about 44°C, and most preferably about 42.5 ± 1°C.

In other preferred embodiments, the aliquot is cooled below normal body temperature such that the mean temperature of the aliquot is within the range of from about 4°C to about 36.5°C, more preferably from about 10°C to about 30°C, and even more preferably from about 15°C to about 25°C

The oxidative environment stressor can be the application to the aliquot of solid, liquid or gaseous oxidizing agents. Preferably, it involves exposing the aliquot to a mixture of medical grade oxygen and ozone gas, most preferably by applying to the aliquot medical grade oxygen gas having ozone as a component therein. The ozone content of the gas stream and the flow rate of the gas stream are preferably selected such that the amount of ozone introduced to the blood aliquot, either on its own or in combination with one of the other stressors, does not give rise to excessive levels of cell damage, and so that, when the treated aliquot is injected into a subject, the desired effect will be achieved. Suitably, the gas stream has an ozone content of up to about 300 µg/ml, preferably up to about 100 µg/ml, more preferably about 30 µg/ml, even more preferably up to about 20 µg/ml, particularly preferably from about 10 µg/ml to about 20 µg/ml, and most preferably about 14.5 ± 1.0µ g/ml. The gas

stream is suitably supplied to the aliquot at a rate of up to about 2.0 litres/min, preferably up to about 0.5 litres/min, more preferably up to about 0.4 litres/min, even more preferably up to about 0.33 litres/min, and most preferably about 0.24 ± 0.024 litres/min. The lower limit of the flow rate of the gas stream is preferably not lower than 0.01 litres/min, more preferably not lower than 0.1 litres/min, and even more preferably not lower than 0.2 litres/min.

The ultraviolet light stressor is suitably applied by irradiating the aliquot under treatment from a source of UV light. Preferred UV sources are UV lamps emitting UV-C band wavelengths, i.e. at wavelengths shorter than about 280 nm. Ultraviolet light corresponding to standard UV-A (wavelengths from about 315 to about 400 nm) and UV-B (wavelengths from about 280 to about 315) sources can also be used. As in the case of the oxidative stressor, the UV dose should be selected, on its own or in combination of the other chosen stressor(s), so that excessive amounts of cell damage do not occur, and so that, when the treated aliquot is injected into a subject, the desired effect will be achieved. For example, an appropriate dosage of such UV light, can be obtained from up to eight lamps arranged to be exposed to the sample container holding the aliquot, operated at an intensity to deliver a total UV light energy at 253.7 nm at the surface of the blood of from about 0.025 to about 10 joules/cm^2, preferably from about 0.1 to about 3.0 joules/cm^2. Such a treatment, applied in combination with the oxidative environment stressor, provides a modified blood aliquot which is ready for injection into the subject.

It is preferred to subject the aliquot to the oxidative environment stressor, the UV light stressor and the temperature stressor simultaneously, following the subjection of the aliquot to the mechanical stress, e.g. by extraction of the blood from the patient. Thus, the aliquot may be maintained at a predetermined temperature above or below body temperature while the oxygen/ozone gas mixture is applied thereto and while it is irradiated with ultraviolet light.

The time for which the aliquot is subjected to the stressors is normally within the time range of from about 0.5 minutes up to about 60 minutes. The time depends to some extent upon the chosen combination of stressors. When UV light is used, the intensity of the UV light may affect the preferred time. The chosen temperature level may also affect the preferred time. When oxidative environment in the form of a gaseous mixture of oxygen and ozone applied to the aliquot is chosen as one of the two stressors, the concentration of the oxidizing agent and the rate at which it is supplied to the aliquot may affect the preferred temperature. Some experimentation to establish optimum times may be necessary on the part of the operator, once the other stressor levels have been set. Under most stressor conditions, preferred times will be in the approximate range of from about 2 to about 5 minutes, more preferably about 3 minutes. The starting blood temperature, and the rate at which it can be warmed or cooled to a predetermined temperature, tends to vary from subject to subject. Warming is suitably by use of one or more infrared lamps placed adjacent to the aliquot container. Other methods of warming can also be adopted.

As noted, it is preferred to subject the aliquot of blood to a mechanical stressor, as well as the chosen stressor(s) discussed above. Extraction of the blood aliquot from the patient through an injection needle constitutes the most convenient way of obtaining the aliquot for further extracorporeal treatment, and this extraction procedure imparts a suitable mechanical stress to the blood aliquot. The mechanical stressor may be supplemented by subsequent processing, for example the additional mechanical shear stress caused by bubbling as the oxidative stressor is applied.

In the practice of the preferred process of the present invention, the blood aliquot may be treated with the heat, UV light and oxidative environment stressors using an apparatus of the type described in aforementioned U.S. Patent No. 4,968,483 to Mueller. The aliquot is placed in a

suitable, sterile container, which is fitted into the machine. A UV-permeable container is used and the UV lamps are switched on for a fixed period before the other stressor is applied, to allow the output of the UV lamps to stabilize. When a temperature stressor is used combination, the UV lamps are typically on while the temperature of the aliquot is adjusted to the predetermined value, e.g. 42.5 ± 1 °C. Four UV lamps are suitably used, placed around the container.

In the preferred method of the invention, a mammalian patient is given one or more courses of treatments, each course of treatment comprising the administration to a mammalian subject of one or more (e.g. one to six) aliquots of mammalian blood modified as discussed above.

For optimum effectiveness of the treatment, it is preferred that no more than one aliquot of modified blood be administered to the subject per day, in one or more injection sites, and that the maximum rest period between any two consecutive aliquots during the course of treatment be no greater than about 21 days. As used herein, the term "rest period" is defined as the number of days between consecutive aliquots or consecutive courses of treatment on which no aliquots of modified blood are administered to the subject.

Therefore, except where aliquots are administered to the subject on consecutive days, a rest period of from 1 to 21 days is provided between any two aliquots during the course of treatment. Moreover, at least one of the rest periods during the course of treatment preferably has a length of about 3 to 15 days.

Although it may be sufficient to administer only one course of treatment as described above to the subject, it may be preferred in some circumstances to administer more than one course of treatment, or to follow the above-described course of treatment by periodic "booster" treatments, if necessary, to maintain the desired effects of the present invention. For

example, it may be preferred to administer booster treatments at intervals of 3 to 4 months following the initial course of treatment, or to administer a second course of treatments to the subject following a rest period of several weeks or months.

The process of the present invention shows potential in the treatment and prophylaxis of a wide variety of T-cell mediated delayed type hypersensitivity reactions, including those mentioned above. In particular, the following conditions show particularly attractive potential for treatment with the process of the invention:

contact hypersensitivity to plant and animal secretions such as poison ivy, poison oak and nettles (urticaria);

eczema;

atopic dermatitis;

erythema multiforma;

angioedema vasculitis;

atopic conjunctivitis;

skin reactions to contact with certain chemicals e.g. nickel, latex, etc., in solid or solution form;

reactions to drug administrations, especially β-lactam antibiotic administration;

protein-induced reactions to food ingestion.

The invention is further illustrated and described with reference to the following specific examples, comprising animal studies conducted in an approved manner.

EXAMPLE 1

The effectiveness of the treatment according to a preferred embodiment of the present invention, on contact hypersensitivity (CHS), was assessed on laboratory mice, according to approved animal experimentation

procedures, using the method described by Kondo et. al., "Lymphocyte function associated antigen-1 (LFA-1) is required for maximum elicitation of allergic contact dematitis" Br J.Dermatol. 131:354-359, 1994, with minor variations.. The disclosure thereof is incorporated herein by reference. Briefly, to induce CHS, the abdominal skin of each mouse was shaved and painted with dinitrodifluorobenzene DNFB, the sensitizing chemical, using 25 µl of 0.5% DNFB in 4:1 acetone:olive oil solution. This sensitization was applied to four groups of five Balb C mice.

Whole blood was obtained from Balb C mice, by extraction from a main artery through an injection needle, and treated with an anti-coagulant. An aliquot of this was subjected to the process of a preferred embodiment of the invention, to obtain treated blood. The remainder was left untreated, for use in control experiments. Since these mice are genetically identical, the administration of the treated blood to others of the group is equivalent to administration of the treated blood to the donor animal.

To obtain treated blood, the selected aliquot, in a sterile, UV-transmissive container, was treated simultaneously with a gaseous oxygen/ozone mixture and ultraviolet light at elevated temperature using an apparatus as generally described in aforementioned U.S.Patent No. 4,968,483 Mueller et.al. Specifically, 10 ml of citrated blood was transferred to a sterile, low density polyethylene vessel (more specifically, a Vasogen VC7002 Blood Container) for ex vivo treatment with stressors according to the invention. Using an apparatus as described in the aforementioned Mueller patent (more specifically, a Vasogen VC7001 apparatus), the blood was heated to 42.5±1°C and at that temperature irradiated with UV light principally at a wavelength of 253.7 nm, while oxygen/ozone gas mixture was bubbled through the blood to provide the oxidative environment and to facilitate exposure of the blood to UV. The constitution of the gas mixture was 14.5 ± 1.0 μg ozone/ml, with the remainder of the mixture comprising medical grade oxygen. The gas mixture was bubbled through the aliquot at a rate of 240 ± 24 ml/min for a period of 3

minutes.

Of the 4 groups of sensitized mice, the first, control group A-1 received no treatment. The second, control group B-1, was treated with physiological saline, 50µl. The third, control group C-1, was sham treated, with 50µl of blood which had been extracted but not treated with the stressors. The fourth, test group D-1, was treated with 50µl of blood subjected to stressors as described above. Treatments, each involving intramuscular injection of 50 µl of the respective liquid, started on the day of sensitization, and was repeated every day for a total of 6 days. On the same day as the last treatment, but after its administration, the animals were challenged with DNFB, by applying to the ears of each animal 10µl of 0.2% solution of DNFB. Inflammation due to CHS manifests itself in a swelling of the ears. Ear thickness was measured, 24 hours after challenge, with a Peacock spring-loaded micrometer (Ozaki Co., Tokyo, Japan). The results were expressed as the change (from pre-challenge level) in ear thickness and represent the mean maximal increase at 24 hours after challenge.

The experiments were repeated two more times, using two more sets of four groups of animals, to ensure statistical significance in the results. Figure 1 of the accompanying drawings is a graphical presentation of these results. A notable and significant reduction in ear thickness (inflammation) is to be observed with the animals treated according to this preferred process of the invention, as compared with any of the other groups. Figure 2 of the accompanying drawings represent photographs of cross-sections of the ears of a representative treated animal of group D-1 (picture (a)) and a representative untreated group A-1 animal (picture(b)). The decreased skin thickness, and the reduced lymphocyte infiltration (lower density of dark stained cells) is readily apparent on picture (a) from the treated animal, further demonstrating a significant reduction in inflammation.

The percentage suppression when compared with the standard

CHS response (no treatment, control group A-1) is 8% for the saline treatment group B-1, 14% for the sham treatment group C-1 and 46% for group D-1, treated according to the embodiment of the process of the invention.

EXAMPLE 2

The procedure of Example was followed, using four groups of Balb/C mice, with one group receiving a blood aliquot which had been subjected to UV and ozone/oxygen bubbling, as described, but without application of the heat stressor (i.e. treated at room temperature). Thus, group A-2 received no treatment, group B-2 received untreated blood (sham treatment), group C-2 received blood treated with UV and ozone but no heat, and group D-2 received blood treated the same way as in the case of group D-1 of Example 1.

The results are presented graphically on Fig. 3, in the same manner as Fig. 1. The result from group D-2 is marginally better than that from group C-2. The percentage suppression when compared to the standard CHS response (no treatment, group A-2) is 9% for group B-2, sham treatment, 52.5% for group C-2 and 54% for group D-2.

EXAMPLE 3

Whole blood was obtained from Balb/C mice. Part of the blood was subjected to UV, ozone and heat treatment as described in Example 1, and part of the blood remained untreated. Both the untreated blood and the treated blood were centrifuged to obtain a cellular fraction, and washed with saline. The treated and untreated fractions were administered to animals challenged with DNFB to develop contact hypersensitivity as described in Example 1.

Four groups of 5 mice each were injected according to the schedule of Example 1, and evaluated, as follows: Group A-3 - no-treatment;

Group B-3 - cellular fraction of sham treated blood; Group C-3 - cellular part of treated blood; Group D-3 - whole treated blood. The administrations to the mice took place just prior to sensitization with 0.5% DNFB and continued every day until challenge with 0.2% DNFB, 5 days later. A total of 6 injections were administered to each mouse.

The ear swelling of each mouse was measured 24 hours after challenge. Each experiment was repeated three times, to ensure statistical significance of the results. Net ear swelling as a measure of contact hypersensitivity and suppression thereof was calculated as 1 - (ear swelling of blood administer mouse/ ear swelling of no blood administered mouse) x 100.

The results are presented graphically on Fig. 4., a summary of three experiments. A significant suppression of CHS is seen with the cellular fraction of the treated blood. There was no significant difference between the treated cellular fraction and treated whole blood.

Although the invention has been described in connection with certain preferred embodiments, it is to be appreciated that it is not limited thereto. Rather, the present invention includes within its scope all embodiments which may fall within the scope of the following claims.

WHAT IS CLAIMED IS:

1. Use for the treatment or prophylaxis of T-cell mediated delayed type hypersensitivity disorders in a mammalian patient, of an aliquot of modified mammalian blood for administration to the patient, the blood aliquot having been modified extracorporeally by simultaneous or sequential exposure to the stress of an oxidative environment and the stress of UV radiation.

2. Use according to claim 1 wherein the stressors are applied substantially simultaneously to the blood aliquot.

3. Use according to claim 1 or claim 2, further including the application of mechanical stress to the aliquot.

4. Use according to claim 1, claim 2 or claim 3 further including the subjection of the blood aliquot to a temperature stress, substantially simultaneously with the application of the UV stress and the oxidative environment stress.

5. Use according to any preceding claim wherein the oxidative environment is a gaseous mixture of oxygen and ozone, bubbled through the aliquot.

6. Use according to claim 4 or claim 5 as appendant to claim 4 wherein the temperature stressor is a temperature in the approximate range 37-55°C.

7. Use according to any preceding claim wherein the aliquot of blood has a volume of from 0.1 - 100 mls.

8. Use according to any preceding claim wherein the oxidative environment is a gas stream of ozone and medical grade oxygen, bubbled through the aliquot, the stream having an ozone content of up to 300 µg per ml, supplied to the aliquot at a rate of up to about 2.0 litres per minute.

9. Use according to any preceding claim wherein the UV stressor is UV light of wavelength shorter than about 280 nm.

10. Use according to any preceding claim wherein the aliquot is subjected to the UV stressor and the oxidative environment stressor for a period of time from about 0.5 to 60 minutes.

11. Use according to any preceding claim wherein the disorder is a contact hypersensitivity.

12. Use according to claim 11 wherein the contact hypersensitivity is a hypersensitivity to a plant secretion.

13. Use according to any of claims 1 - 10 wherein the disorder is selected from eczema, atopic dermatitis, erythema multiforma, angioedema vasculitis, and atopic conjunctivitis.

14. Use according to any of claims 1 - 10 wherein the disorder is contact hypersensitivity skin reaction to chemical solids or solutions.

15. Use according to any of claims 1 - 10 wherein the disorder is a reaction to β-lactam antibiotic administration.

16. Use according to any of claims 1 - 10 wherein the disorder is a protein induced reaction to food ingestion.

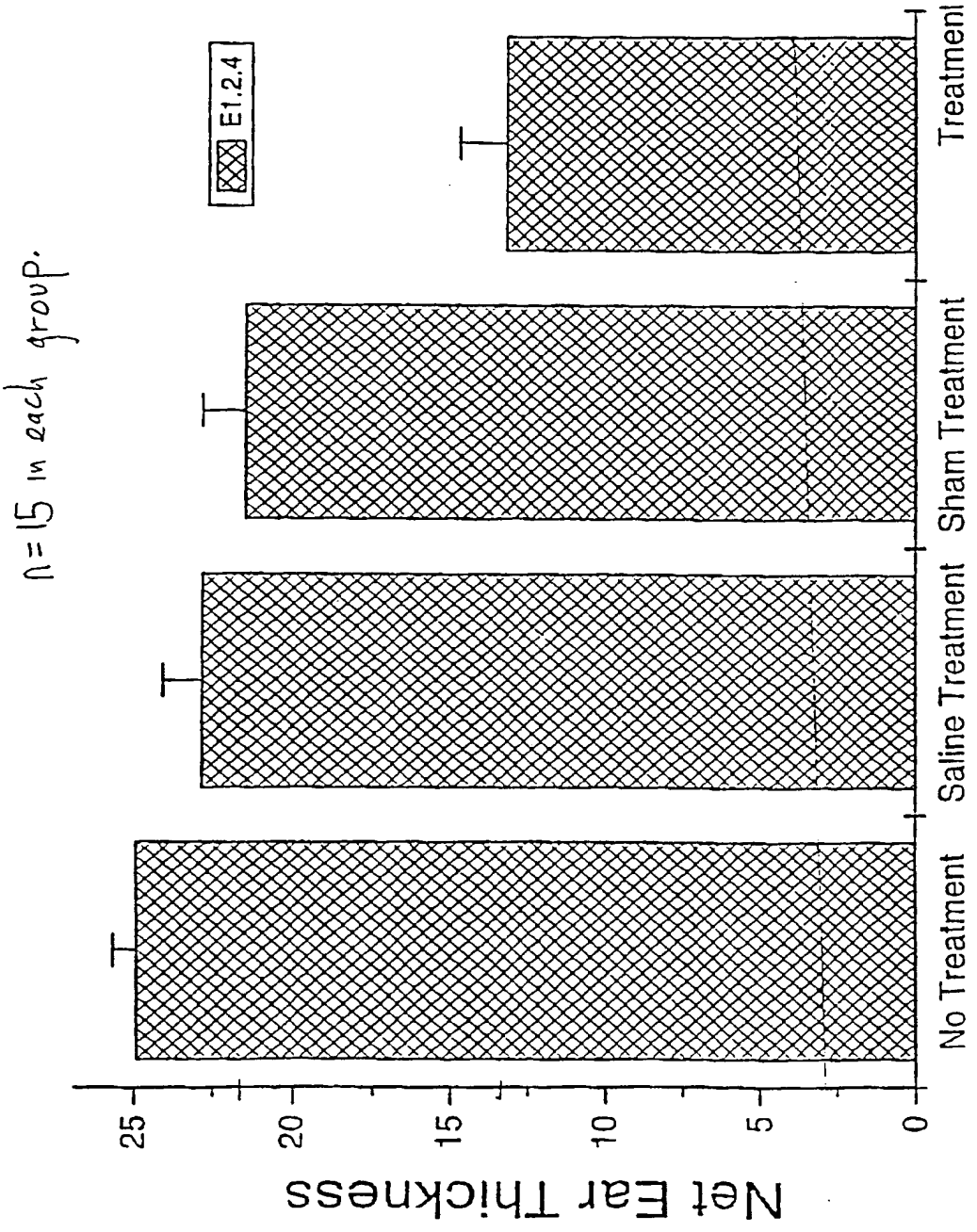

Fig. 1

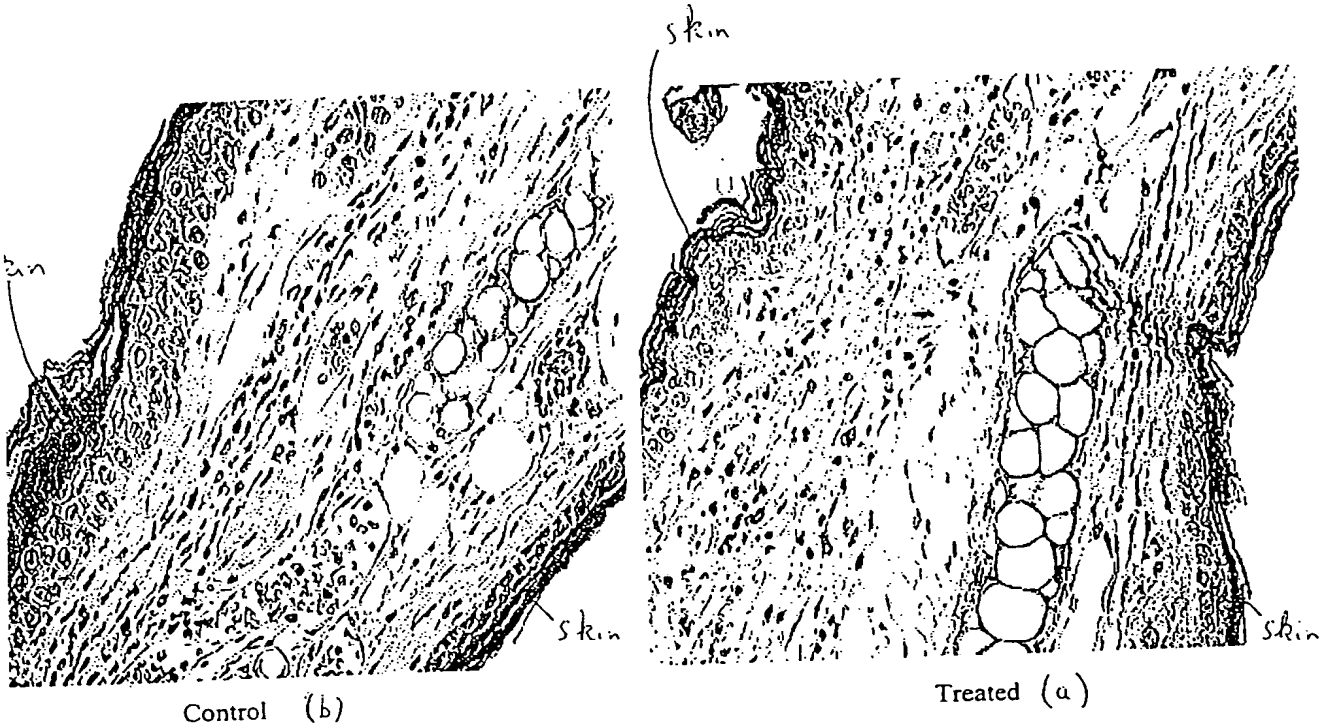

FIG. 2

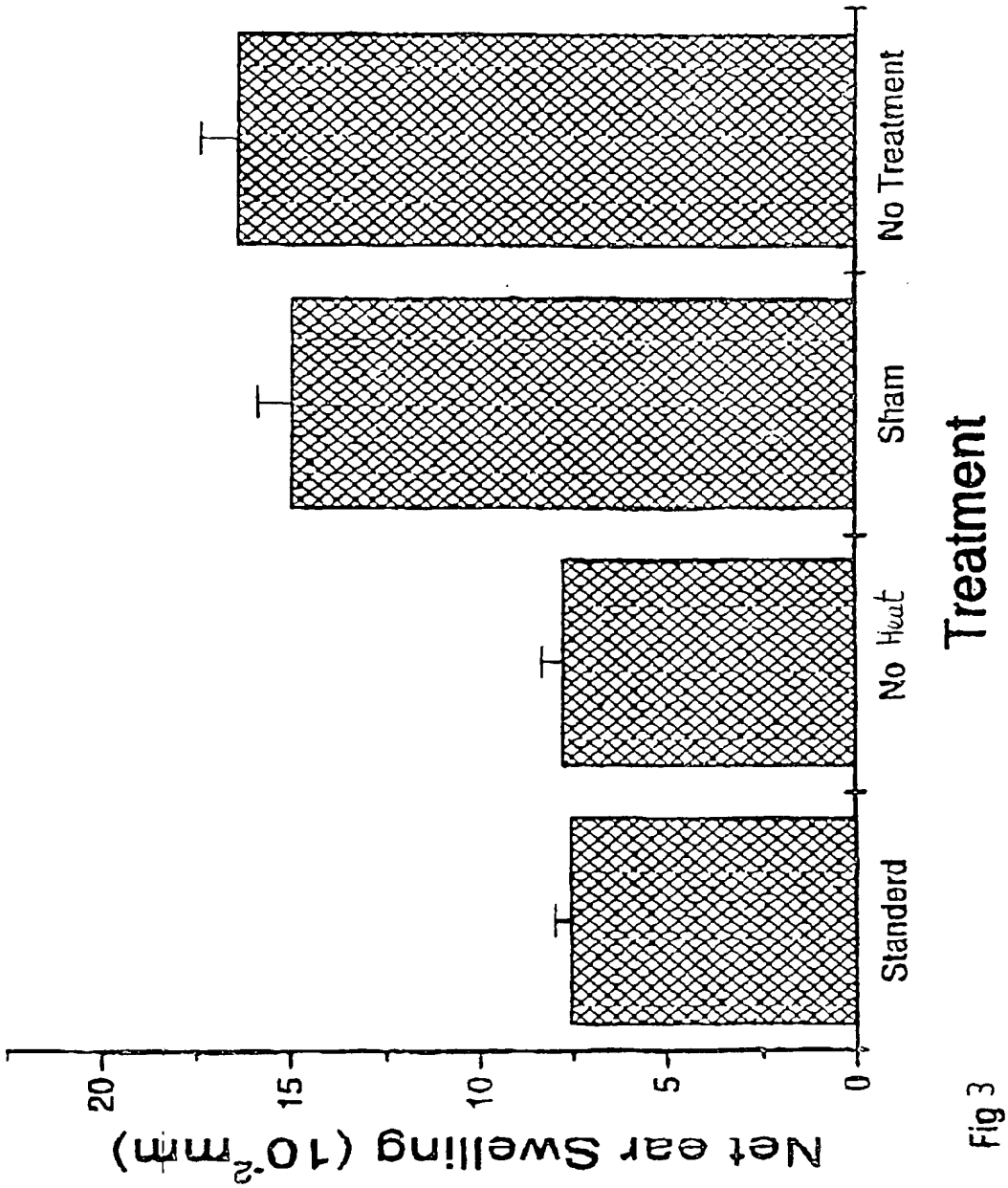

Fig 3

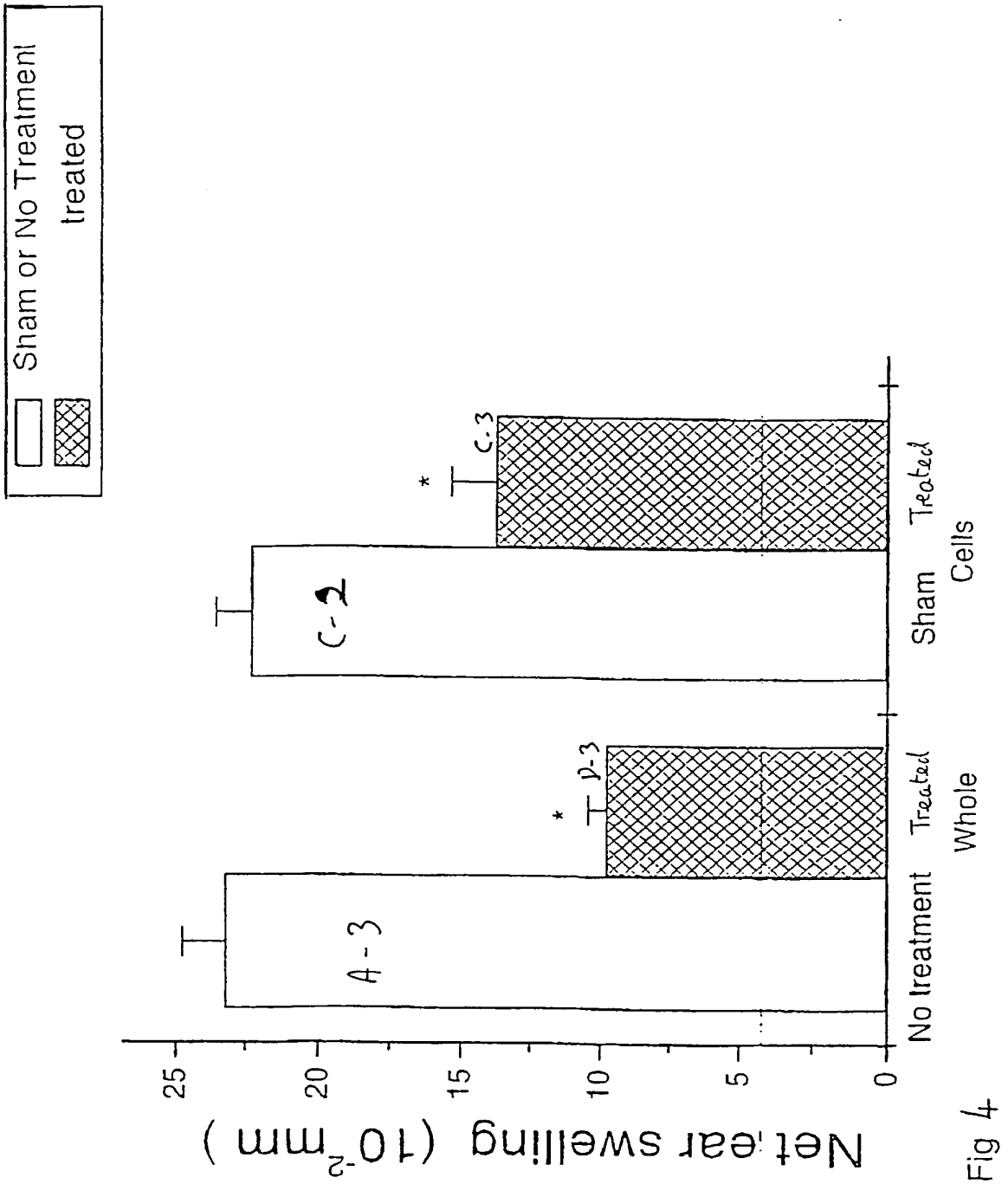

Fig 4

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(54) Title: TREATMENT OF HYPERSENSITIVITY REACTION DISORDERS

(57) Abstract: T-cell mediated delayed type hypersensitivity conditions in mammalian patients are alleviated by a process in which an aliquot of blood is withdrawn from the patient, treated extracorporeally with a combination of UV radiation and an oxidative environment, such as an oxygen/ozone gas mixture bubbled through the aliquot, and then re-injected into the patient.

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Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, WPI Data, EPO-Internal, PAJ, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 07436 A (VASOGEN INC.) 26 February 1998 (1998-02-26) cited in the application page 17, paragraph 4; claims	1-16
A	US 5 147 289 A (R.L. EDELSON) 15 September 1992 (1992-09-15) column 6, line 22 - line 29; claims 1,4,15,16 column 10, line 56 - line 65	1-16

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

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Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Ryckebosch, A

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Form PCT/ISA/210 (second sheet) (July 1992)

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Inte onal Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; 1992 VAN IPEREN H P ET AL: "An animal model for extracorporeal photochemotherapy based on contact hypersensitivity." Database accession no. PREV199395005009 XP002152751 abstract & JOURNAL OF PHOTOCHEMISTRY AND PHOTOBIOLOGY B BIOLOGY, vol. 15, no. 4, 1992, pages 361-366, ISSN: 1011-1344 ---	1-16
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T	G.M. SHIVJI ET AL.: "Effects of VAS972 therapy on allergic contact hypersensitivity" THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, vol. 114, no. 4, April 2000 (2000-04), page 862 XP000960563 BALTIMORE, US abstract nr. 675 ---	1-16
T	G.M. SHIVJI ET AL.: "The effect of VAS972 on allergic contact hypersensitivity" JOURNAL OF CUTANEOUS MEDICINE AND SURGERY, vol. 4, no. 3, July 2000 (2000-07), pages 132-137, XP000960566 the whole document -----	1-16

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Information on patent family members

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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US 5147289 A	15-09-1992	US 5383847 A	24-01-1995

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(54) Title: TREATMENT OF HYPERSENSITIVITY REACTION DISORDERS

(57) Abstract: T-cell mediated delayed type hypersensitivity conditions in mammalian patients are alleviated by a process in which an aliquot of blood is withdrawn from the patient, treated extracorporeally with a combination of UV radiation and an oxidative environment, such as an oxygen/ozone gas mixture bubbled through the aliquot, and then re-injected into the patient.

TREATMENT OF HYPERSENSITIVITY REACTION DISORDERS

FIELD OF THE INVENTION

This invention relates to the field of medicine and medical treatments. In particular, the invention relates to improved methods and compositions for treatment and prophylaxis of T-cell mediated delayed type hypersensitivity reactions in mammalian patients, involving introduction into the patient of a small amount of treated, modified mammalian blood.

BACKGROUND OF THE INVENTION

T-cell mediated delayed type hypersensitivity reactions as the term is used herein means adverse reactions on the part of a mammalian patient to foreign agents, commonly but not exclusively manifested by development of skin disorders on the patient, and in which the disorder takes at least 24 hours to exhibit full manifestation. Many of these are diagnosable by skin tests. The reactions may be chemical contact reactions, food ingestion reactions or drug ingestion reactions. Specific examples of such conditions include contact hypersensitivity reactions, in which the skin of the patient exhibits a reaction to an agent which the body has previously encountered, by contact or by inoculation. The "poison ivy" type of reaction is a specific example of contact hypersensitivity. Hypersensitivity to β-lactam antibiotics (e.g. penicillins) is an example where inoculation of a foreign agent gives rise to a skin disorder-manifested, T-cell mediated delayed type hypersensitivity. The external agents can be plant, animal, insect or reptilian secretions, chemical or biochemical antigens, from synthetic or natural sources. Various types of fibers, fabrics and the like, such as latex used in surgical gloves, can give rise to T-cell mediated hypersensitivity reaction in certain individuals. The offending external agents can be water-borne agents such as dissolved salts and minerals, encountered for example in environmental, mining, metallurgical and chemical manufacturing operations. T-cell mediated delayed type hypersensitivity

reactions are to be distinguished from psoriasis, which is an autoimmune disorder which manifests itself in red scaly skin patches having an inflammatory component, but not resulting from contact reaction.

Mammalian blood modified by exposure simultaneously to certain stressors has been reported to be useful for the treatment of a variety of pathological conditions. The stressors to which the blood is exposed are an oxidative environment namely ozone/oxygen gas mixtures applied to the blood, a temperature stressor and UV light. Thus:

U.S. Patent No. 4,968,483 Mueller et al. describes an apparatus for oxygenating blood by treating an aliquot of a patient's blood extracorporeally, with an oxygen/ozone mixture and ultraviolet light, at a controlled temperature. The apparatus taught by Mueller is proposed for use in hematological oxidation therapy.

U.S. Patent No. 5,591,457 Bolton discloses a method of inhibiting the aggregation of blood platelets in a human, a method of stimulating the immune system and a method of treating peripheral vascular diseases such as Raynaud's disease, by extracting an aliquot of blood from a patient, subjecting it to an ozone/oxygen gas mixture and ultraviolet radiation at a temperature in the range of about 37 to 43°C, and then re-injecting the treated blood in the human patient.

International Patent Application PCT/GB93/00259 Bolton describes a process for increasing the content of nitric oxide in the blood of a mammalian subject, potentially useful in treating conditions such as high blood pressure in mammalian subjects, by subjecting a sample of the patient's blood extracorporeally to three stressors simultaneously, namely an ozone/oxygen gas mixture bubbled through the blood sample, exposure to UV radiation and an elevated temperature, followed by re-injection of the treated blood sample into the patient.

International Publication No. WO 98/07436 describes an autoimmune vaccine for administration to human patients to alleviate the symptoms of autoimmune diseases such as rheumatoid arthritis. The vaccine comprises an aliquot of the subject's blood which has been subjected extracorporeally to an oxidizing environment, UV radiation and elevated temperature, simultaneously.

International Publication No. WO 96/34613 relates to treatment of vascular disorders associated with deficient endothelial function, in a mammalian subject, by administration to the patient of an aliquot of blood which has been modified by having been subjected simultaneously to stressors namely elevated temperature in the range of 37° to 55°C, ultraviolet radiation and an oxidative environment

SUMMARY OF THE INVENTION

According to one aspect, the present invention provides use for treatment or prophylaxis of T-cell mediated delayed type hypersensitivity disorders in a mammalian patient, of modified mammalian blood for administration to the patient, the blood having been modified extracorporeally by simultaneous or sequential exposure to the stress of an oxidative environment and the stress of UV radiation.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying FIGURES are presentations of the results of specific Examples described below.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

According to a preferred process of the present invention, an aliquot of blood is extracted from a mammalian subject, preferably a human,

and the aliquot of blood is treated ex vivo, simultaneously or sequentially, with the aforementioned stressors. Then it is injected back into the same subject. Preferably a combination of both of the aforementioned stressors is used.

Preferably also, the aliquot of blood is in addition subjected to mechanical stress. Such mechanical stress is suitably that applied to the aliquot of blood by extraction of the blood aliquot through a conventional blood extraction needle, or a substantially equivalent mechanical stress, applied shortly before the other chosen stressors are applied to the blood aliquot. This mechanical stress may be supplemented by the mechanical stress exerted on the blood aliquot by bubbling gases through it, such as ozone/oxygen mixtures, as described below. Optionally also, a temperature stressor may be applied to the blood aliquot, simultaneously or sequentially with the other stressors, i.e. a temperature at, above or below body temperature.

The terms "aliquot", "aliquot of blood" or similar terms used herein include whole blood, separated cellular fractions of the blood including platelets, separated non-cellular fractions of the blood including plasma, plasma components and combinations thereof. Preferably, in human patients, the volume of the aliquot is up to about 400 ml, preferably from about 0.1 to about 100 ml, more preferably from about 1 to about 15 ml, even more preferably from about 8 to about 12 ml, and most preferably about 10 ml. The effect of the combination of stressors is to modify the blood, and/or the cellular or non-cellular fractions thereof, contained in the aliquot. The modified aliquot is then re-introduced into the subject's body by any suitable method, most preferably intramuscular injection, but also including subcutaneous injection, intraperitoneal injection, intra-arterial injection, intravenous injection and oral administration.

The optionally applied temperature stressor either warms the aliquot being treated to a temperature above normal body temperature or cools the aliquot below normal body temperature. The temperature is selected so

that the temperature stressor does not cause excessive hemolysis in the blood contained in the aliquot and so that, when the treated aliquot is injected into a subject, the desired effect will be achieved. Preferably, the temperature stressor is applied so that the temperature of all or a part of the aliquot is up to about 55°C, and more preferably in the range of from about -5°C to about 55°C.

In some preferred embodiments of the invention, the temperature of the aliquot is raised above normal body temperature, such that the mean temperature of the aliquot does not exceed a temperature of about 55°C, more preferably from about 40°C to about 50°C, even more preferably from about 40°C to about 44°C, and most preferably about 42.5 ± 1°C.

In other preferred embodiments, the aliquot is cooled below normal body temperature such that the mean temperature of the aliquot is within the range of from about 4°C to about 36.5°C, more preferably from about 10°C to about 30°C, and even more preferably from about 15°C to about 25°C

The oxidative environment stressor can be the application to the aliquot of solid, liquid or gaseous oxidizing agents. Preferably, it involves exposing the aliquot to a mixture of medical grade oxygen and ozone gas, most preferably by applying to the aliquot medical grade oxygen gas having ozone as a component therein. The ozone content of the gas stream and the flow rate of the gas stream are preferably selected such that the amount of ozone introduced to the blood aliquot, either on its own or in combination with one of the other stressors, does not give rise to excessive levels of cell damage, and so that, when the treated aliquot is injected into a subject, the desired effect will be achieved. Suitably, the gas stream has an ozone content of up to about 300 µg/ml, preferably up to about 100 µg/ml, more preferably about 30 µg/ml, even more preferably up to about 20 µg/ml, particularly preferably from about 10 µg/ml to about 20 µg/ml, and most preferably about 14.5 ± 1.0µ g/ml. The gas

stream is suitably supplied to the aliquot at a rate of up to about 2.0 litres/min, preferably up to about 0.5 litres/min, more preferably up to about 0.4 litres/min, even more preferably up to about 0.33 litres/min, and most preferably about 0.24 ± 0.024 litres/min. The lower limit of the flow rate of the gas stream is preferably not lower than 0.01 litres/min, more preferably not lower than 0.1 litres/min, and even more preferably not lower than 0.2 litres/min.

The ultraviolet light stressor is suitably applied by irradiating the aliquot under treatment from a source of UV light. Preferred UV sources are UV lamps emitting UV-C band wavelengths, i.e. at wavelengths shorter than about 280 nm. Ultraviolet light corresponding to standard UV-A (wavelengths from about 315 to about 400 nm) and UV-B (wavelengths from about 280 to about 315) sources can also be used. As in the case of the oxidative stressor, the UV dose should be selected, on its own or in combination of the other chosen stressor(s), so that excessive amounts of cell damage do not occur, and so that, when the treated aliquot is injected into a subject, the desired effect will be achieved. For example, an appropriate dosage of such UV light, can be obtained from up to eight lamps arranged to be exposed to the sample container holding the aliquot, operated at an intensity to deliver a total UV light energy at 253.7 nm at the surface of the blood of from about 0.025 to about 10 joules/cm^2, preferably from about 0.1 to about 3.0 joules/cm^2. Such a treatment, applied in combination with the oxidative environment stressor, provides a modified blood aliquot which is ready for injection into the subject.

It is preferred to subject the aliquot to the oxidative environment stressor, the UV light stressor and the temperature stressor simultaneously, following the subjection of the aliquot to the mechanical stress, e.g. by extraction of the blood from the patient. Thus, the aliquot may be maintained at a predetermined temperature above or below body temperature while the oxygen/ozone gas mixture is applied thereto and while it is irradiated with ultraviolet light.

The time for which the aliquot is subjected to the stressors is normally within the time range of from about 0.5 minutes up to about 60 minutes. The time depends to some extent upon the chosen combination of stressors. When UV light is used, the intensity of the UV light may affect the preferred time. The chosen temperature level may also affect the preferred time. When oxidative environment in the form of a gaseous mixture of oxygen and ozone applied to the aliquot is chosen as one of the two stressors, the concentration of the oxidizing agent and the rate at which it is supplied to the aliquot may affect the preferred temperature. Some experimentation to establish optimum times may be necessary on the part of the operator, once the other stressor levels have been set. Under most stressor conditions, preferred times will be in the approximate range of from about 2 to about 5 minutes, more preferably about 3 minutes. The starting blood temperature, and the rate at which it can be warmed or cooled to a predetermined temperature, tends to vary from subject to subject. Warming is suitably by use of one or more infrared lamps placed adjacent to the aliquot container. Other methods of warming can also be adopted.

As noted, it is preferred to subject the aliquot of blood to a mechanical stressor, as well as the chosen stressor(s) discussed above. Extraction of the blood aliquot from the patient through an injection needle constitutes the most convenient way of obtaining the aliquot for further extracorporeal treatment, and this extraction procedure imparts a suitable mechanical stress to the blood aliquot. The mechanical stressor may be supplemented by subsequent processing, for example the additional mechanical shear stress caused by bubbling as the oxidative stressor is applied.

In the practice of the preferred process of the present invention, the blood aliquot may be treated with the heat, UV light and oxidative environment stressors using an apparatus of the type described in aforementioned U.S. Patent No. 4,968,483 to Mueller. The aliquot is placed in a

suitable, sterile container, which is fitted into the machine. A UV-permeable container is used and the UV lamps are switched on for a fixed period before the other stressor is applied, to allow the output of the UV lamps to stabilize. When a temperature stressor is used combination, the UV lamps are typically on while the temperature of the aliquot is adjusted to the predetermined value, e.g. 42.5 ± 1 °C. Four UV lamps are suitably used, placed around the container.

In the preferred method of the invention, a mammalian patient is given one or more courses of treatments, each course of treatment comprising the administration to a mammalian subject of one or more (e.g. one to six) aliquots of mammalian blood modified as discussed above.

For optimum effectiveness of the treatment, it is preferred that no more than one aliquot of modified blood be administered to the subject per day, in one or more injection sites, and that the maximum rest period between any two consecutive aliquots during the course of treatment be no greater than about 21 days. As used herein, the term "rest period" is defined as the number of days between consecutive aliquots or consecutive courses of treatment on which no aliquots of modified blood are administered to the subject.

Therefore, except where aliquots are administered to the subject on consecutive days, a rest period of from 1 to 21 days is provided between any two aliquots during the course of treatment. Moreover, at least one of the rest periods during the course of treatment preferably has a length of about 3 to 15 days.

Although it may be sufficient to administer only one course of treatment as described above to the subject, it may be preferred in some circumstances to administer more than one course of treatment, or to follow the above-described course of treatment by periodic "booster" treatments, if necessary, to maintain the desired effects of the present invention. For

example, it may be preferred to administer booster treatments at intervals of 3 to 4 months following the initial course of treatment, or to administer a second course of treatments to the subject following a rest period of several weeks or months.

The process of the present invention shows potential in the treatment and prophylaxis of a wide variety of T-cell mediated delayed type hypersensitivity reactions, including those mentioned above. In particular, the following conditions show particularly attractive potential for treatment with the process of the invention:

contact hypersensitivity to plant and animal secretions such as poison ivy, poison oak and nettles (urticaria);

eczema;

atopic dermatitis;

erythema multiforma;

angioedema vasculitis;

atopic conjunctivitis;

skin reactions to contact with certain chemicals e.g. nickel, latex, etc., in solid or solution form;

reactions to drug administrations, especially β-lactam antibiotic administration;

protein-induced reactions to food ingestion.

The invention is further illustrated and described with reference to the following specific examples, comprising animal studies conducted in an approved manner.

EXAMPLE 1

The effectiveness of the treatment according to a preferred embodiment of the present invention, on contact hypersensitivity (CHS), was assessed on laboratory mice, according to approved animal experimentation

procedures, using the method described by Kondo et. al., "Lymphocyte function associated antigen-1 (LFA-1) is required for maximum elicitation of allergic contact dematitis" Br J.Dermatol. 131:354-359, 1994, with minor variations.. The disclosure thereof is incorporated herein by reference. Briefly, to induce CHS, the abdominal skin of each mouse was shaved and painted with dinitrodifluorobenzene DNFB, the sensitizing chemical, using 25 µl of 0.5% DNFB in 4:1 acetone:olive oil solution. This sensitization was applied to four groups of five Balb C mice.

Whole blood was obtained from Balb C mice, by extraction from a main artery through an injection needle, and treated with an anti-coagulant. An aliquot of this was subjected to the process of a preferred embodiment of the invention, to obtain treated blood. The remainder was left untreated, for use in control experiments. Since these mice are genetically identical, the administration of the treated blood to others of the group is equivalent to administration of the treated blood to the donor animal.

To obtain treated blood, the selected aliquot, in a sterile, UV-transmissive container, was treated simultaneously with a gaseous oxygen/ozone mixture and ultraviolet light at elevated temperature using an apparatus as generally described in aforementioned U.S.Patent No. 4,968,483 Mueller et.al. Specifically, 10 ml of citrated blood was transferred to a sterile, low density polyethylene vessel (more specifically, a Vasogen VC7002 Blood Container) for ex vivo treatment with stressors according to the invention. Using an apparatus as described in the aforementioned Mueller patent (more specifically, a Vasogen VC7001 apparatus), the blood was heated to 42.5± 1 °C and at that temperature irradiated with UV light principally at a wavelength of 253.7 nm, while oxygen/ozone gas mixture was bubbled through the blood to provide the oxidative environment and to facilitate exposure of the blood to UV. The constitution of the gas mixture was 14.5 ± 1.0 µg ozone/ml, with the remainder of the mixture comprising medical grade oxygen. The gas mixture was bubbled through the aliquot at a rate of 240 ± 24 ml/min for a period of 3

minutes.

Of the 4 groups of sensitized mice, the first, control group A-1 received no treatment. The second, control group B-1, was treated with physiological saline, 50µl. The third, control group C-1, was sham treated, with 50µl of blood which had been extracted but not treated with the stressors. The fourth, test group D-1, was treated with 50µl of blood subjected to stressors as described above. Treatments, each involving intramuscular injection of 50 µl of the respective liquid, started on the day of sensitization, and was repeated every day for a total of 6 days. On the same day as the last treatment, but after its administration, the animals were challenged with DNFB, by applying to the ears of each animal 10µl of 0.2% solution of DNFB. Inflammation due to CHS manifests itself in a swelling of the ears. Ear thickness was measured, 24 hours after challenge, with a Peacock spring-loaded micrometer (Ozaki Co., Tokyo, Japan). The results were expressed as the change (from pre-challenge level) in ear thickness and represent the mean maximal increase at 24 hours after challenge.

The experiments were repeated two more times, using two more sets of four groups of animals, to ensure statistical significance in the results. Figure 1 of the accompanying drawings is a graphical presentation of these results. A notable and significant reduction in ear thickness (inflammation) is to be observed with the animals treated according to this preferred process of the invention, as compared with any of the other groups. Figure 2 of the accompanying drawings represent photographs of cross-sections of the ears of a representative treated animal of group D-1 (picture (a)) and a representative untreated group A-1 animal (picture(b)). The decreased skin thickness, and the reduced lymphocyte infiltration (lower density of dark stained cells) is readily apparent on picture (a) from the treated animal, further demonstrating a significant reduction in inflammation.

The percentage suppression when compared with the standard

CHS response (no treatment, control group A-1) is 8% for the saline treatment group B-1, 14% for the sham treatment group C-1 and 46% for group D-1, treated according to the embodiment of the process of the invention.

EXAMPLE 2

The procedure of Example was followed, using four groups of Balb/C mice, with one group receiving a blood aliquot which had been subjected to UV and ozone/oxygen bubbling, as described, but without application of the heat stressor (i.e. treated at room temperature). Thus, group A-2 received no treatment, group B-2 received untreated blood (sham treatment), group C-2 received blood treated with UV and ozone but no heat, and group D-2 received blood treated the same way as in the case of group D-1 of Example 1.

The results are presented graphically on Fig. 3, in the same manner as Fig. 1. The result from group D-2 is marginally better than that from group C-2. The percentage suppression when compared to the standard CHS response (no treatment, group A-2) is 9% for group B-2, sham treatment, 52.5% for group C-2 and 54% for group D-2.

EXAMPLE 3

Whole blood was obtained from Balb/C mice. Part of the blood was subjected to UV, ozone and heat treatment as described in Example 1, and part of the blood remained untreated. Both the untreated blood and the treated blood were centrifuged to obtain a cellular fraction, and washed with saline. The treated and untreated fractions were administered to animals challenged with DNFB to develop contact hypersensitivity as described in Example 1.

Four groups of 5 mice each were injected according to the schedule of Example 1, and evaluated, as follows: Group A-3 - no-treatment;

Group B-3 - cellular fraction of sham treated blood; Group C-3 - cellular part of treated blood; Group D-3 - whole treated blood. The administrations to the mice took place just prior to sensitization with 0.5% DNFB and continued every day until challenge with 0.2% DNFB, 5 days later. A total of 6 injections were administered to each mouse.

The ear swelling of each mouse was measured 24 hours after challenge. Each experiment was repeated three times, to ensure statistical significance of the results. Net ear swelling as a measure of contact hypersensitivity and suppression thereof was calculated as 1 - (ear swelling of blood administer mouse/ ear swelling of no blood administered mouse) x 100.

The results are presented graphically on Fig. 4., a summary of three experiments. A significant suppression of CHS is seen with the cellular fraction of the treated blood. There was no significant difference between the treated cellular fraction and treated whole blood.

Although the invention has been described in connection with certain preferred embodiments, it is to be appreciated that it is not limited thereto. Rather, the present invention includes within its scope all embodiments which may fall within the scope of the following claims.

WHAT IS CLAIMED IS:

1. Use for the treatment or prophylaxis of T-cell mediated delayed type hypersensitivity disorders in a mammalian patient, of an aliquot of modified mammalian blood for administration to the patient, the blood aliquot having been modified extracorporeally by simultaneous or sequential exposure to the stress of an oxidative environment and the stress of UV radiation.

2. Use according to claim 1 wherein the stressors are applied substantially simultaneously to the blood aliquot.

3. Use according to claim 1 or claim 2, further including the application of mechanical stress to the aliquot.

4. Use according to claim 1, claim 2 or claim 3 further including the subjection of the blood aliquot to a temperature stress, substantially simultaneously with the application of the UV stress and the oxidative environment stress.

5. Use according to any preceding claim wherein the oxidative environment is a gaseous mixture of oxygen and ozone, bubbled through the aliquot.

6. Use according to claim 4 or claim 5 as appendant to claim 4 wherein the temperature stressor is a temperature in the approximate range 37-55°C.

7. Use according to any preceding claim wherein the aliquot of blood has a volume of from 0.1 - 100 mls.

8. Use according to any preceding claim wherein the oxidative environment is a gas stream of ozone and medical grade oxygen, bubbled through the aliquot, the stream having an ozone content of up to 300 µg per ml, supplied to the aliquot at a rate of up to about 2.0 litres per minute.

9. Use according to any preceding claim wherein the UV stressor is UV light of wavelength shorter than about 280 nm.

10. Use according to any preceding claim wherein the aliquot is subjected to the UV stressor and the oxidative environment stressor for a period of time from about 0.5 to 60 minutes.

11. Use according to any preceding claim wherein the disorder is a contact hypersensitivity.

12. Use according to claim 11 wherein the contact hypersensitivity is a hypersensitivity to a plant secretion.

13. Use according to any of claims 1 - 10 wherein the disorder is selected from eczema, atopic dermatitis, erythema multiforma, angioedema vasculitis, and atopic conjunctivitis.

14. Use according to any of claims 1 - 10 wherein the disorder is contact hypersensitivity skin reaction to chemical solids or solutions.

15. Use according to any of claims 1 - 10 wherein the disorder is a reaction to β-lactam antibiotic administration.

16. Use according to any of claims 1 - 10 wherein the disorder is a protein induced reaction to food ingestion.

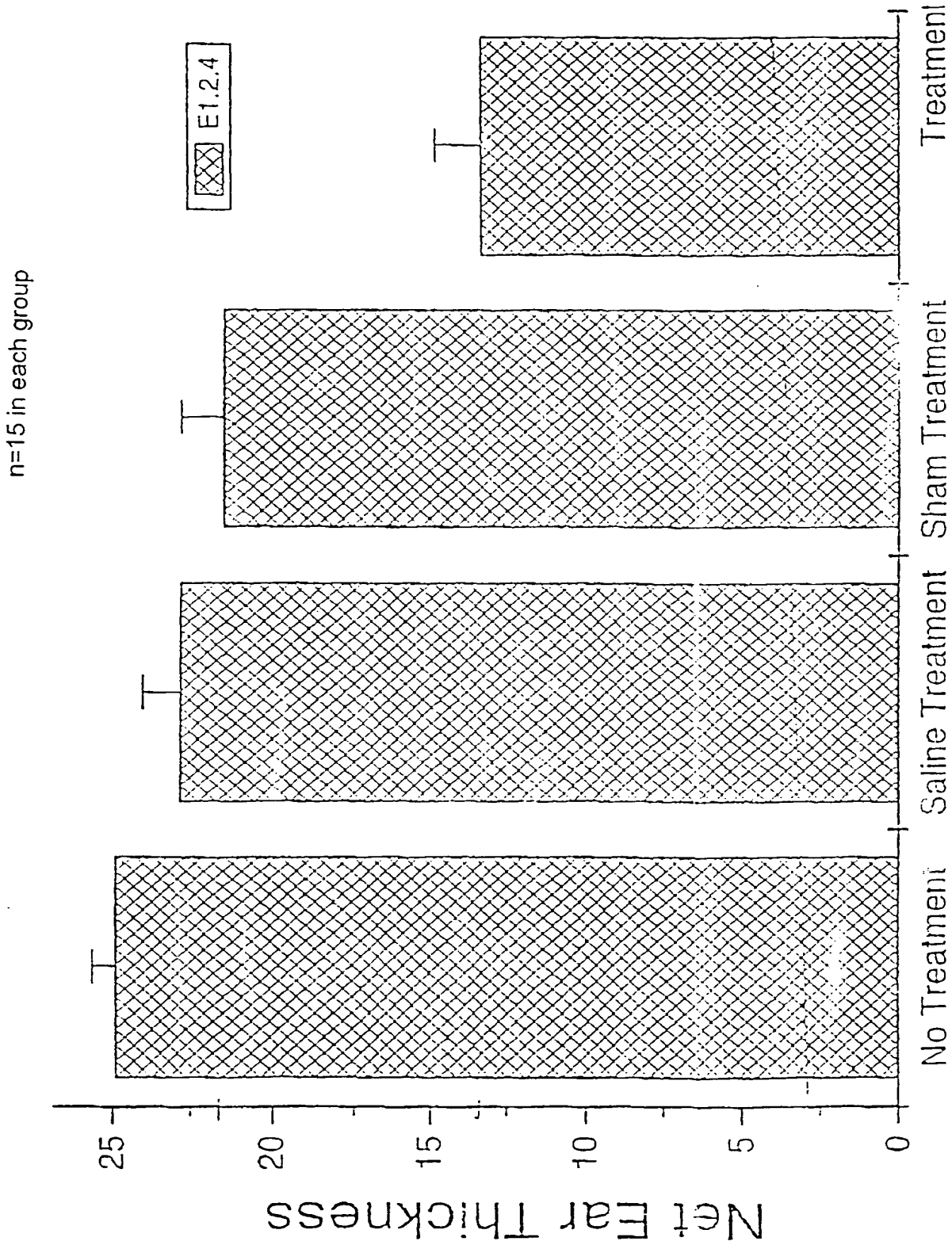

FIGURE 1

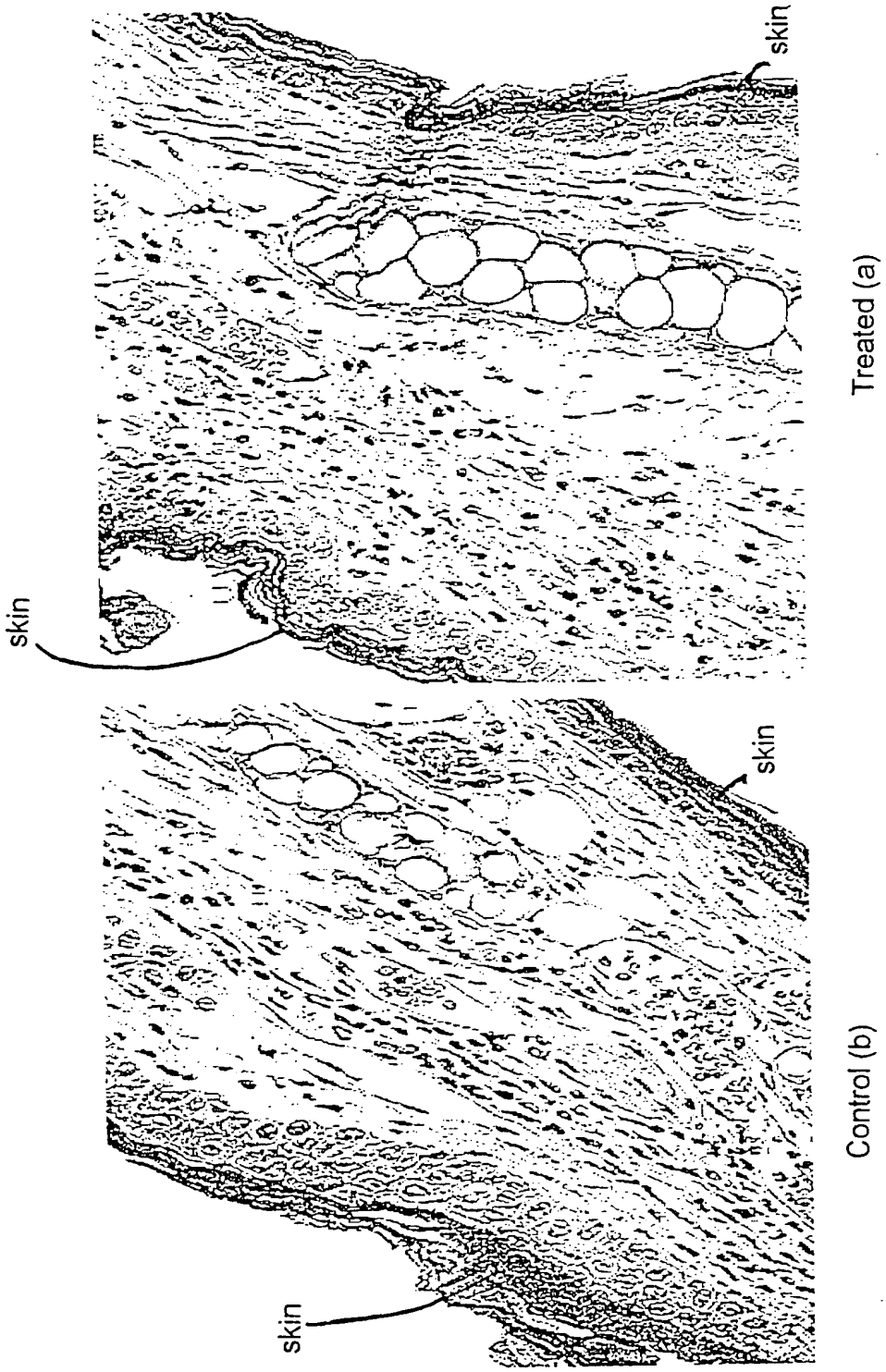

FIGURE 2

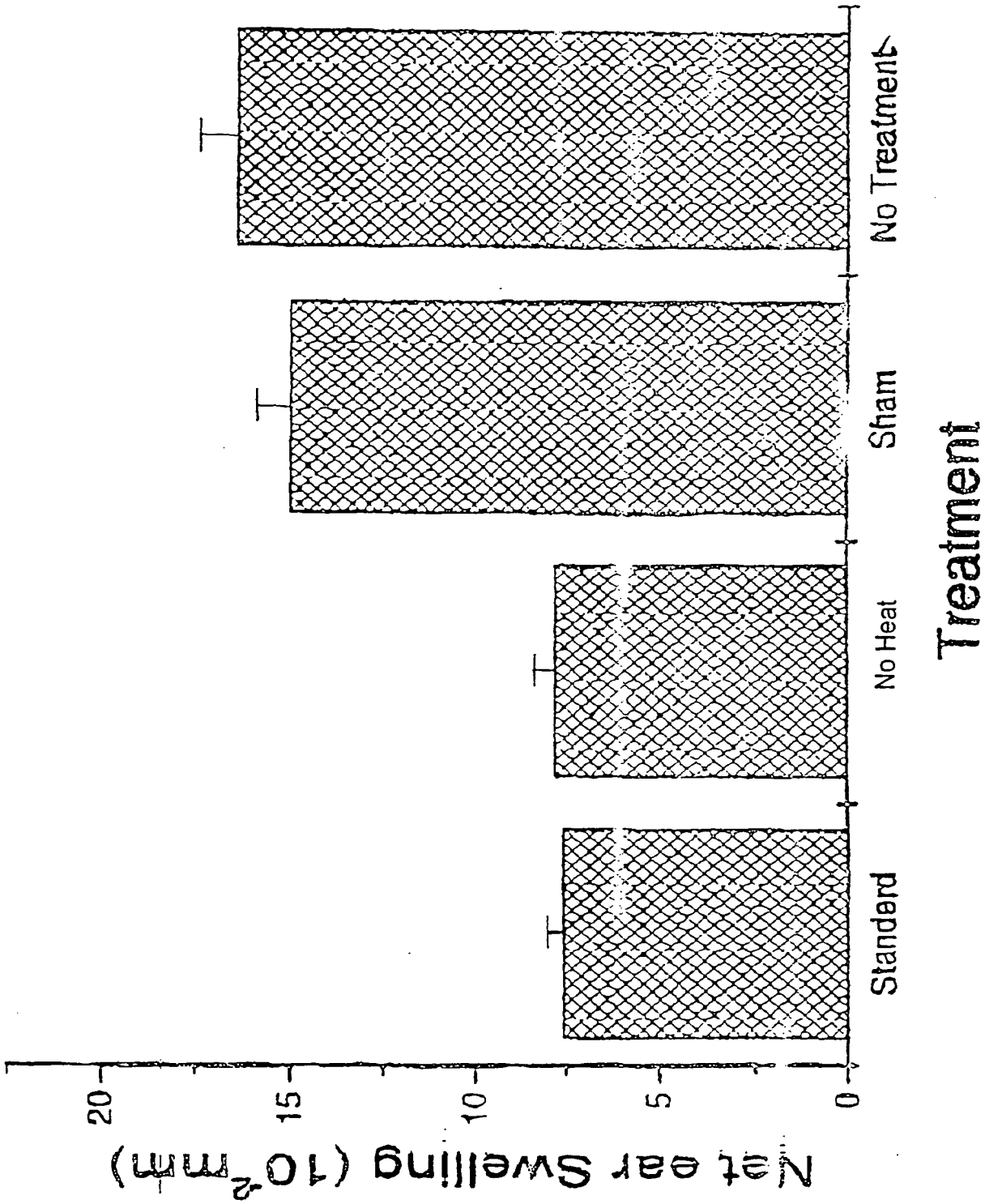

FIGURE 3

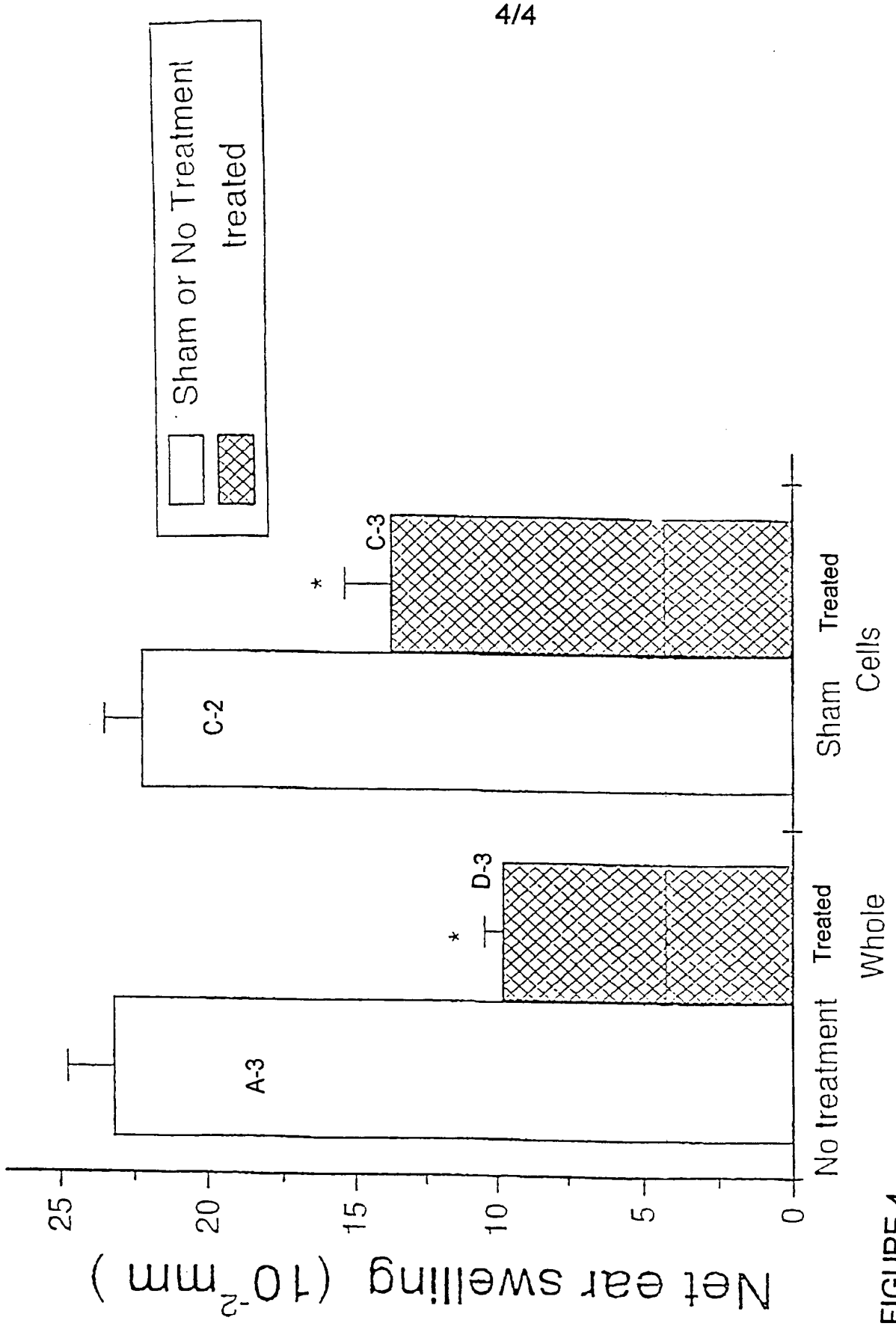

FIGURE 4

INTERNATIONAL SEARCH REPORT

Inter[illegible]onal Application No
PCT/CA 00/00433

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K35/14 A61K41/00 A61P37/08 A61P17/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A61M

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, WPI Data, EPO-Internal, PAJ, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 07436 A (VASOGEN INC.) 26 February 1998 (1998-02-26) cited in the application page 17, paragraph 4; claims	1-16
A	US 5 147 289 A (R.L. EDELSON) 15 September 1992 (1992-09-15) column 6, line 22 - line 29; claims 1,4,15,16 column 10, line 56 - line 65	1-16

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Date of the actual completion of the international search	Date of mailing of the international search report
14 November 2000	24/11/2000

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/CA 00/00433

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; 1992 VAN IPEREN H P ET AL: "An animal model for extracorporeal photochemotherapy based on contact hypersensitivity." Database accession no. PREV199395005009 XP002152751 abstract & JOURNAL OF PHOTOCHEMISTRY AND PHOTOBIOLOGY B BIOLOGY, vol. 15, no. 4, 1992, pages 361-366, ISSN: 1011-1344 ---	1-16
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T	G.M. SHIVJI ET AL.: "Effects of VAS972 therapy on allergic contact hypersensitivity" THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, vol. 114, no. 4, April 2000 (2000-04), page 862 XP000960563 BALTIMORE, US abstract nr. 675 ---	1-16
T	G.M. SHIVJI ET AL.: "The effect of VAS972 on allergic contact hypersensitivity" JOURNAL OF CUTANEOUS MEDICINE AND SURGERY, vol. 4, no. 3, July 2000 (2000-07), pages 132-137, XP000960566 the whole document -----	1-16

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter[illegible]onal Application No
PCT/CA 00/00433

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